

**Please replace the paragraph starting page 6, line 7, with the paragraph below.**

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I<sup>1</sup> Figure 5 shows the cleavage of purified extracellular matrix (ECM) proteins. Figure 5A shows that after five minutes of protease incubation with VN, degradation products could not be identified by Coomassie blue staining. Figure 5B shows that incubation of FN with the protease for up to 12 hours did not result in formation of additional degradation products. Figure 5C shows that no significant cleavage of human laminin was observed under the experimental conditions assayed.

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**Please replace the paragraph starting page 6, line 9 with the paragraph below.**

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I<sup>2</sup> Figure 6 shows the induction of cytopathic effect and fibronectin cleavage in human umbilical vein endothelial cells (HUVEC) cultures. Figure 6A shows that western immunoblot analysis of cells in the absence of protease, or treated with boiled protease for up to 8 hours, showed no detectable FN degradation. Figure 6B shows that by three hours after protease addition, zones of clearing occurred in the cell monolayer.

B. In response to Examiner comments on sequence submission informalities, SEQ ID numbers have been added to the specification where appropriate.

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**Please substitute the paragraph starting on page 32, line 13, starting "To create a stable zymogen....," and ending on page 33, line 12 with the below paragraph. Please note the correction of the three letter code Gln on page 32, line 21.**

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I<sup>3</sup> To create a stable zymogen to facilitate crystallographic studies and generate enzymatically deficient or inactive protease for structure-function studies, mutant forms of the cysteine protease protein are made and characterized. A targeted mutagenesis scheme creates changes that: (i) disrupt protease activity; (ii) prevent zymogen processing; (iii) prevent substrate binding; and (iv) alter immunoreactivity. Amino acids are changed to structurally neutral alanine. A mutant protein that lacks protease activity, but which retains

I<sup>3</sup>

antigenicity, is generated by mutagenesis of the single cysteine residue (Cys-192->Ala-192) at the catalytic site of the molecule. Also, His-340 and Gln-185 and Asn-356 are mutagenized. These three changes are epistatic to the Cys-192 mutation, but may alone exhibit altered activity. Trp-357, thought to be involved in substrate binding and similarly positioned within papain, will also be targeted. A stable zymogen precursor is also created by mutating residues surrounding the protease cleavage site at Lys-145. In addition, mutagenesis of Cys-192 may prevent autoproteolysis, as occurred for a Cys->Ser mutant of papain, the prototype cysteine protease. Other mutagenesis targets include a putative nucleotide binding domain (SEQ ID NO:17) (GVGKVG) and a potential collagen docking region (SEQ ID NO:18) [(GXX)<sub>3</sub>] within carboxy terminal portion of the protein. Site-directed mutagenesis is used, by the charged-to-alanine-scanning method, to substitute positively and negatively charged amino acids (often involved in recognition and activity) with alanine. Many of the charged residues (14 lysine, 7 arginine, 12 aspartate, and 7 glutamate residues in the mature peptide) are expected to lie on the surface of the cysteine protease structure, and some are expected to define epitopes on the molecule. In particular, a region of charged amino acids, from 307 to 321 (8/15 charged), is examined; this region includes the site of *speB2* and *speB4* amino acid substitutions. Residues in antigenic regions identified in the epitope mapping studies are also mutated.

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